CBIC Control Number

363432

Lubrizol

The Lubrizol Corporation
29400 Lakeland Boulevard
Wickliffe, OH 44092-2298
Phone: 440-347-5122
Fax: 440-347-4295

January 13, 2015

TSCA Confidential Business Information Center (7407M)
WJC East - Room 6428; Attn: Section 8(e)
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460-0001

Subject: TSCA Section 8(e) Submission of an Audited Draft Report of a Skin Sensitization Study of OS329036A [CAS No.: 220755-49-7; Chemical Name: Benzenesulfonic acid, Mono-C20-24-alkyl Derivs., Sodium Salts] in Guinea Pigs.

Dear Sir/Madam:

The Lubrizol Corporation is submitting the audited draft report titled, *A Sensitization Study of OS329036A by Dermal Administration in Guinea Pigs – Modified Buehler Design* pursuant to Section 8(e) Substantial Risk reporting requirements under the Toxic Substance Control Act. The results of this report indicate that this substance causes skin sensitization in the guinea pig. This study was conducted to assess the skin sensitization potential of this substance and to support REACH registration.

This submission does not contain confidential business information.

Sincerely,

Leonard Sweet, PhD, MPH
Manager Global Toxicology
Product Safety & Compliance

charles river

A Sensitization Study of OS329036A by Dermal Administration in Guinea Pigs-Modified Buehler Design

Test Guidelines
OECD (406), EPA-OPPTS (870.2600)

Author
Jason W. Smedley, BS

Study Completion Date

Performing Laboratory
Charles River Laboratories
Preclinical Services, Ohio (PCS-OH)
640 North Elizabeth Street
Spencerville, OH 45887
United States

Sponsor
The Lubrizol Corporation
29400 Lakeland Blvd.
Wickliffe, OH 44092-2298
United States

Laboratory Project ID
20061359

Page Count
1 of 82

COMPLIANCE STATEMENT AND REPORT APPROVAL

This study was performed in accordance with the United States Code of Federal Regulations, Title 40, Parts 792: Good Laboratory Practice (GLP) Standards and as accepted by Regulatory Authorities throughout the European Union (OECD Principles of Good Laboratory Practice) and Japan (MAFF and METI), and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

Exceptions from the above regulations are listed below.

- Characterization of the test substance was performed by the Sponsor or Sponsor subcontractor according to established SOPs, controls, and approved test methodologies to ensure integrity and validity of the results generated; these analyses were not conducted in compliance with the GLP or Good Manufacturing Practice (GMP) regulations.
- Concentration, stability, and homogeneity of the test substance formulations were not determined in this study.
- Concentration, stability, and homogeneity of the α-Hexylcinnamaldehyde (HCA) formulations were not determined in this study.

This study was conducted in accordance with the procedures described herein. All deviations authorized/acknowledged by the Study Director are documented in the Study Records. The report represents an accurate and complete record of the results obtained.

There were no deviations from the above regulations that affected the overall integrity of the study or the interpretation of the study results and conclusions.

Study Director: _____ Date: _____

Typed Name of Signer: Jason W. Smedley, BS

Typed Name of Company: Charles River Laboratories, Preclinical Services, OH

Sponsor: _____ Date: _____

Typed Name of Signer: _____

Typed Name of Company: The Lubrizol Corporation

Submitter: _____ Date: _____

Typed Name of Signer: _____

Typed Name of Company: _____

QUALITY ASSURANCE STATEMENT

Protocol: 20061359

This Study has been audited by the Quality Assurance Unit in accordance with the applicable Good Laboratory Practice regulations. Reports were submitted in accordance with SOPs as follows:

QA INSPECTION DATES

		Dates Findings Submitted to:	
Date(s) of Audit	**Phase(s) Audited**	**Study Director**	**Study Director Management**
25-Sep-2014	Final Protocol	26-Sep-2014	26-Sep-2014
30-Sep-2014	Dose Preparation	30-Sep-2014	30-Sep-2014
07-Oct-2014	Dose Administration	07-Oct-2014	07-Oct-2014
18-Dec-2014	Protocol Amendment 1	19-Dec-2014	19-Dec-2014
18-Dec-2014	Data Review - Formulations	19-Dec-2014	19-Dec-2014
18-Dec-2014	Data Review - Technical Operations	19-Dec-2014	19-Dec-2014
18-Dec-2014	Data Review - Necropsy	19-Dec-2014	19-Dec-2014
19-Dec-2014	Draft Report	19-Dec-2014	19-Dec-2014

In addition to the above-mentioned audits, process-based and/or routine facility inspections were also conducted during the course of this study. Inspection findings, if any, specific to this study were reported by the Quality Assurance Unit to the Study Director and Management and listed as a Phase Audit on this Quality Assurance Statement.

The Quality Assurance Statements for the work conducted at the Test Sites were reviewed and are included in the appropriate section of this report.

The Final Report has been reviewed to assure that it accurately describes the materials and methods, and that the reported results accurately reflect the raw data.

Richardson, Krista
Charles River Laboratories

Date

TABLE OF CONTENTS

COMPLIANCE STATEMENT AND REPORT APPROVAL....2
QUALITY ASSURANCE STATEMENT....3
TABLE OF CONTENTS....4
LIST OF TABLES....6
LIST OF APPENDICES....7
1. RESPONSIBLE PERSONNEL....8
1.1. Testing Facility....8
2. SUMMARY....9
2.1. OS329036A....9
2.2. α-Hexylcinnamaldehyde (HCA)....9
2.3. Conclusion....10
3. INTRODUCTION....11
4. MATERIALS AND METHODS....11
4.1. Test and Control Substances....11
4.1.1. Test Substance....11
4.1.2. Control Substance(s)....11
4.2. Positive Control....12
4.2.1. Positive Control Substance Components....12
4.3. Test Substance Characterization....12
4.4. Analysis of Test Substance....13
4.5. Reserve Samples....13
4.6. Test Substance Inventory and Disposition....13
4.7. Dose Formulation and Analysis....13
4.7.1. Preparation of Test Substance....13
4.7.2. HCA Preparation....13
4.8. Test System....13
4.8.1. Receipt....13
4.8.2. Justification for Test System and Number of Animals....13
4.8.3. Animal Identification....14
4.8.4. Environmental Acclimation....14
4.8.5. Selection, Assignment, and Disposition of Animals....14
4.8.6. Husbandry....14
4.9. Experimental Design – Range-Finding Phase....15
4.9.1. Justification of Route and Dose Levels....16
4.9.2. Administration of Test Materials....16
4.10. In-life Procedures, Observations, and Measurements – Range-Finding Phases....16
4.10.1. Mortality/Moribundity Checks....16
4.10.2. Clinical Observations....16
4.10.3. Body Weights....17
4.10.4. Scheduled Euthanasia....17
4.11. Experimental Design – Main Phase....17

4.11.1. Justification of Route and Dose Levels 17
4.11.2. Administration of Test Materials 18
4.12. In-life Procedures, Observations, and Measurements 19
4.12.1. Mortality/Moribundity Checks 19
4.12.2. Clinical Observations 19
4.12.3. Detailed Clinical Observations 19
4.12.4. Dermal Observations 19
4.12.5. Body Weights 20
4.12.6. Unscheduled Deaths 20
4.12.7. Scheduled Euthanasia 20
5. COMPUTERIZED SYSTEMS 20
6. STATISTICAL ANALYSIS 20
7. RETENTION OF RECORDS, SAMPLES, AND SPECIMENS 20
8. RESULTS 22
8.1. Mortality 22
8.2. Range-Finding Phase 22
8.3. Main Phase 22
8.3.1. Induction Phase 22
8.3.2. Challenge Phase 22
8.3.3. Rechallenge Phase 23
8.4. Body Weights 23
8.5. Clinical and Necropsy Observations 23
9. CONCLUSION 24
10. SCIENTIFIC REPORT REVIEW 25
11. REFERENCES 26

LIST OF TABLES

Table 1 Topical Range-Finding Data 27
Table 2 Second Topical Range-Finding Data 29
Table 3 Individual Induction Data 31
Table 4 Individual Challenge Data 34
Table 5 Individual Rechallenge Data 39

LIST OF APPENDICES

Appendix 1 Protocol and Protocol Amendment 42
Appendix 2 Test Substance Characterization 69
Appendix 3 Dermal Grading System 71
Appendix 4 Individual Body Weight Data 77
Appendix 5 Individual Clinical/Necropsy Observations 81

1. RESPONSIBLE PERSONNEL

1.1. Testing Facility

Study Director	Jason W. Smedley, BS
Site Director	Rusty E. Rush, MS, DABT
Director of Research	Mark A. Morse, PhD, DABT
Study Coordinator/Supervisor, Study Coordination	Kelly L. Landin, BS
Staff Veterinarian	Lynn M. Lucke, DVM
Director, Operations	Todd N. Merriman, BS, MBA, LATG
Supervisor, Formulations	Beth A. Hoover, BS, MS, MBA, CPhT
Study Supervisor, In-Life	Andrew J. Slaughterbeck, BS, ALAT
Primary Technician	William H. Maus III
Manager, Report Coordination	Cheryl A. Bellamy, BS
Lead Archivist	Rebecca R. English, BS

2. SUMMARY

The dermal sensitization potential of OS329036A was evaluated in Hartley-derived albino guinea pigs. Ten male and 10 female guinea pigs were topically treated with 75% OS329036A in mineral oil once per week, for 3 consecutive weeks. Following a 2-week rest period, a challenge was performed whereby the 20 test and 10 previously untreated (naïve) challenge control guinea pigs were topically treated with 35% OS329036A in mineral oil. Challenge responses in the test animals were higher than those of the challenge control animals. Following a 1-week rest period, a rechallenge was performed in which the 20 test and 10 previously untreated (naïve) rechallenge control guinea pigs were topically treated with 15% OS329036A in mineral oil. Rechallenge responses in the test animals were higher than those of the control animals.

An α-Hexylcinnamaldehyde (HCA) positive control group consisting of 10 HCA test and 10 HCA control guinea pigs was included in this study. The animals were treated as above with the HCA test animals receiving 5% w/v HCA in ethanol for induction and 2.5% and 1.0% w/v HCA in acetone for challenge.

2.1. OS329036A

Following challenge with 35% OS329036A, dermal scores of 2 were noted in 18/20 test animals at the 24-hour scoring interval. At the 48-hour scoring interval, dermal scores of 2 were noted in 16/20 test animals. The remaining test animals had scores of 1 at the 24 and 48 hour scoring intervals. Dermal reactions in the challenge control animals were scores of 0, ±, or 1. Group mean dermal scores were higher in the test animals (1.8 to 1.9) as compared to challenge control animals (0.6).

Following rechallenge with 15% OS329036A in mineral oil, dermal scores of 2 were noted in 14/20 test animals at the 24- and 48-hour scoring intervals. Dermal reactions in the remaining test animals were ± or 1. Dermal reactions in the rechallenge control animals were scores of 0, ±, or 1. Group mean dermal scores were higher in the test animals (1.7) as compared to rechallenge control animals (0.7 to 0.8).

2.2. α-Hexylcinnamaldehyde (HCA)

Following challenge with 2.5% w/v HCA in acetone, dermal scores of 2 were noted in 10/10 HCA test animals at the 24-hour and 48-hour scoring intervals. Dermal reactions in the HCA control animals were limited to scores of 0. Group mean dermal scores were higher in the HCA test animals (2.0) compared to the HCA control animals (0.0).

Following challenge with 1.0% w/v HCA in acetone, dermal scores of 1 or 2 were noted in 10/10 HCA test animals at the 24-hour and 48-hour scoring intervals. Dermal reactions in the HCA control animals were limited to scores of 0. Group mean dermal scores were higher in the HCA test animals (1.7 to 1.8) compared to the HCA control animals (0.0).

2.3. Conclusion

Based on the results of this study, OS329036A is considered to be a contact sensitizer in guinea pigs, as the criterion for sensitization (dermal scores ≥ 2 in at least 15% of the test animals) was met. The results of the HCA positive control study demonstrated that a valid test was performed and indicated that the test design would detect potential contact sensitizers.

3. INTRODUCTION

The objective of this study was to assess the dermal sensitization potential of OS329036A when given as multiple topical applications to guinea pigs.

The Study Director signed the protocol on 15 Sep 2014 and dosing was initiated on 07 Oct 2014. The in-life phase of the study was completed on 14 Nov 2014. The experimental start dates were 23 Sep 2014 (OECD) and 30 Sep 2014 (EPA), the experimental completion date was 14 Nov 2014 (OECD/EPA). The study protocol and protocol amendment are presented in Appendix 1.

Prior to initiation of the main sensitization study, a topical range-finding study was conducted in guinea pigs to aid in the selection of dosage levels. The in-life phase of the range-finding study was initiated with test substance administration on 30 Sep 2014 and concluded on 02 Oct 2014. A second topical range-finding study was conducted prior to challenge. The in-life phase of the second range-finding study was initiated with test substance administration on 28 Oct 2014 and concluded on 30 Oct 2014.

4. MATERIALS AND METHODS

4.1. Test and Control Substances

4.1.1. Test Substance

Identification:	OS329036A
Batch (Lot) No.:	OS329036A
Receipt Date:	23 Sep 2014
Expiration Date:	23 Sep 2016
Physical Description:	Viscous brown liquid
Purity:	100%
Storage Conditions:	Kept in a controlled room temperature area
Supplier:	Lubrizol

4.1.2. Control Substance(s)

Identification:	Mineral oil, light, NF
Batch (Lot) No.:	142705
Receipt Date:	18 Sep 2014
Expiration Date:	18 Sep 2015
Physical Description:	Clear colorless solution
Storage Conditions:	Kept in a room temperature area
Supplier:	Fisher Scientific

4.2. Positive Control

Identification: 5.0% (w/v) HCA in Ethanol

Identification: 2.5% (w/v) HCA in Acetone

Identification: 1.0% (w/v) HCA in Acetone

4.2.1. Positive Control Substance Components

Identification: α-Hexylcinnamaldehyde (HCA)

- Batch (Lot) No.: FDZEJ
- Receipt Date: 10 Apr 2014
- Expiration Date: 10 Apr 2015
- Physical Description: Clear yellow liquid
- Purity: 93.6%
- Storage Conditions: Kept in a room temperature area, protected from light, desiccated
- Supplier: TCI America

Identification: Ethanol

- Batch (Lot) No.: CB1960
- Receipt Date: 25 Nov 2013
- Expiration Date: 11 Nov 2016
- Physical Description: Clear colorless liquid
- Storage Conditions: Kept in a room temperature area in a flammable cabinet
- Supplier: Pharmco-AAPER

Identification: Acetone

- Batch (Lot) No.: 143440
- Receipt Date: 01 Jul 2014
- Expiration Date: 01 Jul 2015
- Physical Description: Clear colorless liquid
- Storage Conditions: Kept in a room temperature area in a flammable cabinet
- Supplier: Fisher Scientific

4.3. Test Substance Characterization

The Sponsor provided to the Testing Facility documentation of the identity, strength, purity, composition, and stability for the test substance. A Certificate of Analysis was provided to the Testing Facility and is presented in Appendix 2.

4.4. Analysis of Test Substance

The stability of the bulk test substance was not determined during the course of this study.

4.5. Reserve Samples

A reserve sample was collected for each batch (lot) of test substance (1 mL), control substance (1 mL), and positive control substance components (HCA, ethanol, and acetone; 1 g or 1 mL) and maintained under the appropriate storage conditions by the Testing Facility.

4.6. Test Substance Inventory and Disposition

Records of the receipt, distribution, storage, and disposition of the test substance (including empty containers) were maintained. With the exception of reserve samples, all unused Sponsor-supplied bulk test substance will be returned to the Sponsor (after issuance of the final reports of all studies using this material). All empty containers were maintained for the duration of the study.

4.7. Dose Formulation and Analysis

4.7.1. Preparation of Test Substance

The test substance, OS329036A, was administered as received and/or diluted with the control substance on the day of dosing during the range-finding phases, during induction, challenge, and rechallenge. Selected doses were achieved by adjustment of test substance concentration in the control substance. Details of the preparation and dispensing of the test substance have been retained in the Study Records.

4.7.2. HCA Preparation

HCA dosing formulations were prepared at appropriate concentrations to meet dose level requirements. The dosing formulations were prepared, protected from light, and dispensed on the day of dosing. Details of the preparation and dispensing of the positive control substance have been retained in the Study Records.

4.8. Test System

4.8.1. Receipt

On 23 Sep 2014, 44 male and 44 female Hartley-derived albino guinea pigs were received from Charles River Laboratories, Stone Ridge, NY. The animals were examined and weighed on the day following receipt.

4.8.2. Justification for Test System and Number of Animals

The Hartley-derived guinea pig was chosen as the animal model for this study as it is an accepted rodent species for preclinical toxicity testing by regulatory agencies.

The total number of animals used in this study was considered to be the minimum required to properly characterize the effects of the test substance. This study was designed such that it did not require an unnecessary number of animals to accomplish its objectives.

At this time, studies in laboratory animals provide the best available basis for extrapolation to humans and are required to support regulatory submissions. Acceptable models which do not use live animals currently do not exist.

4.8.3. Animal Identification

Each animal was identified by a cage card and plastic ear tag.

4.8.4. Environmental Acclimation

The animals were acclimated to their designated housing for at least 7 days before the first day of dosing.

4.8.5. Selection, Assignment, and Disposition of Animals

The animals chosen for study were arbitrarily selected from healthy animals. All animals received a detailed pretest observation prior to dosing. Only healthy animals were chosen for study use.

The male range-finding animals were approximately 5 weeks of age on the day prior to dosing with body weights of 334 grams and 345 grams. The female range-finding animals were approximately 5 weeks of age on the day prior to dosing with body weights of 325 grams and 331 grams.

The male main phase animals were approximately 6 weeks of age on the day prior to Induction 1 dosing with body weights ranging from 335 grams to 443 grams. The female main phase animals were approximately 6 weeks of age on the day prior to Induction 1 dosing with body weights ranging from 349 grams to 402 grams.

The male second range-finding animals were approximately 9 weeks of age on the day prior to dosing with body weights of 530 grams and 556 grams. The female second range-finding animals were approximately 9 weeks of age on the day prior to dosing with body weights of 496 grams and 502 grams.

The disposition of all animals was documented in the study records.

4.8.6. Husbandry

4.8.6.1. Housing

The animals were pair housed (2 animals of the same sex and same dosing group together) throughout the study in polycarbonate cages containing direct bedding material. As an alternative, guinea pigs were individually housed in solid bottom cages containing a hiding device and direct bedding material. Housing and care were as specified in the USDA Animal Welfare Act (9 CFR, Parts 1, 2, and 3) and as described in the *Guide for the Care and Use of Laboratory Animals* from the National Research Council.[1]

4.8.6.2. Environmental Conditions

Temperatures of 71°F to 72°F (22°C) with a relative humidity of 49% to 58% were maintained. A 12-hour light/12-hour dark cycle was maintained, except when interrupted for designated

procedures. Ten or greater air changes per hour with 100% fresh air (no air recirculation) were maintained in the animal rooms.

4.8.6.3. Food

PMI Nutrition International Certified Guinea Pig Chow No. 5026 was provided ad libitum throughout the study, except during designated procedures. The feed was analyzed by the supplier for nutritional components and environmental contaminants. Results of the dietary analyses were provided by the supplier for each lot of diet and are on file at the Testing Facility. Based on these results, there were no known contaminants in the feed that would interfere with the objectives of the study.

4.8.6.4. Water

Municipal tap water after treatment by reverse osmosis and ultraviolet irradiation was freely available to each animal via an automatic watering system, except during designated procedures. The water is analyzed semi-annually for microbial contamination and for total dissolved solids, hardness, and various environmental contaminants. Results of these analyses are maintained on file at the Testing Facility. Based on the results of the most recent analysis, there were no contaminants in the water that could interfere with the outcome of the study.

4.8.6.5. Animal Enrichment

Beginning at receipt, guinea pigs were pair housed in solid bottom cages containing direct bedding material. As an alternative, guinea pigs were individually housed in solid bottom cages containing direct bedding material. When individually housed, a hiding comfort device (PVC pipe) was provided. In addition, a timothy hay cube was provided to each animal at least weekly.

4.8.6.6. Veterinary Care

Veterinary care was available throughout the study and the animals were examined by the veterinary staff as warranted by clinical signs or other changes. No veterinary medicinal treatments were administered during the study.

4.9. Experimental Design – Range-Finding Phase

Text Table 1
Experimental Design for the Range-Finding Phase

No. of Animals		Test Material	Site	Patch Design[a]	Dose Volume (mL)	Dose Level Concentration (%)
Males	Females					
2	2	OS329036A	1	25 mm Hilltop Chamber	0.3	100[b]
			2	25 mm Hilltop Chamber	0.3	75[c]
			3	25 mm Hilltop Chamber	0.3	50[c]
			4	25 mm Hilltop Chamber	0.3	25[c]

[a] Occlusive patch.
[b] As received.
[c] The vehicle used was mineral oil.

Text Table 2
Experimental Design for the Second Range-Finding Phase

No. of Animals		Test Material	Site	Patch Design[a]	Dose Volume (mL)	Dose Level Concentration (%)
Males	Females					
2	2	OS329036A	1	25 mm Hilltop Chamber	0.3	35[b]
			2	25 mm Hilltop Chamber	0.3	25[b]
			3	25 mm Hilltop Chamber	0.3	15[b]
			4	25 mm Hilltop Chamber	0.3	5[b]

[a] Occlusive patch.
[b] The vehicle used was mineral oil.

4.9.1. Justification of Route and Dose Levels

The dermal route of exposure was selected because this is the intended route of human exposure.

Four graded levels were utilized for this procedure. Optimally, the range-finding study should produce no systemic toxicity and a spectrum of dermal responses that include Grades 0, ±, 1, and 2 unless the test substance was not dermally irritating at 100%.

4.9.2. Administration of Test Materials

On the day prior to dosing, the guinea pigs selected for the topical range-finding study were weighed and the hair removed from the right and left side of the animals with a small animal clipper. Care was taken to avoid abrading the skin during the clipping procedures.

On Day 0, 4 concentrations of the test substance were prepared and a 0.3 mL dose of each concentration was applied to the clipped area of each topical range-finding animal. The closed chambers (25 mm Hill Top Chamber for each concentration) were applied to the clipped surface as quickly as possible. The trunk of the animal was wrapped with elastic wrap to prevent removal of the chambers and the animal was returned to its cage.

Approximately 6 hours after chamber application, the binding materials were removed. The test sites were then wiped 2 times with gauze moistened in mineral oil, followed by dry gauze, and then wiped with gauze moistened in reverse osmosis deionized (RODI) water, followed by dry gauze, to remove test substance residue and the animals were returned to their cages.

4.10. In-life Procedures, Observations, and Measurements – Range-Finding Phases

The in-life procedures, observations, and measurements listed below were performed for all range-finding animals.

4.10.1. Mortality/Moribundity Checks

The animals were observed for general health/mortality and moribundity twice daily, once in the morning and afternoon, throughout the study.

4.10.2. Clinical Observations

4.10.2.1. Detailed Clinical Observations

The animals were removed from the cage and examined in detail before dosing on Day 0.

4.10.2.2. Dermal Observations

The test sites of each topical range-finding animal were graded for irritation at approximately 24 hours and 48 hours after chamber application using the Macroscopic Dermal Grading System in Appendix 3 according to Buehler.[2]

4.10.3. Body Weights

Each topical range-finding animal was weighed on the day prior to dosing (Day -1).

4.10.4. Scheduled Euthanasia

Following the 48-hour scoring interval, all range-finding animals were euthanized by carbon dioxide inhalation and discarded.

4.11. Experimental Design – Main Phase

Text Table 3
Experimental Design for the Main Phase

Group	No. of Animals		Phase/Treatment			
	Males	Females	Induction 1 to 3	Challenge	Rechallenge	Second Rechallenge[a]
Test	10	10	OS329036A (75%)[b]	OS329036A (35%)[b]	OS329036A (15%)[b]	-
Challenge Control	5	5	-	OS329036A (35%)[b]		-
Rechallenge Control	5	5	-	-	OS329036A (15%)[b]	-
Second Rechallenge Control	5	5	-	-	-	-
HCA Test	5	5	5.0% HCA[c]	2.5% and 1.0% HCA[d]	-	-
HCA Control	5	5	-	2.5% and 1.0% HCA[d]	-	-

Note; - = not applicable.
[a] Second rechallenge was not performed as rechallenge results were definitive.
[b] The vehicle used was mineral oil.
[c] The vehicle used was ethanol.
[d] The vehicle used was acetone.

4.11.1. Justification of Route and Dose Levels

The dermal route of exposure was selected because this is a potential route of human exposure.

The dose concentration for the main induction phase was based upon the results of the range-finding portion of the study. A second range-finding phase was performed to determine dose levels for the challenge phase. The test substance concentration used for challenge should produce no systemic toxicity and dermal responses generally consist of Grades 0 to ±. The results of the challenge procedure were not conclusive; therefore, a rechallenge phase was

conducted to clarify challenge responses. The test substance concentration used for rechallenge should produce no systemic toxicity and dermal responses generally consisting of Grades 0 to ±.

4.11.2. Administration of Test Materials

On the day prior to dosing, the guinea pigs selected for the main study had the hair removed with a small animal clipper. Care was taken to avoid abrading the skin during the clipping procedures.

On the following day, a 0.3 mL dose of the appropriate test or positive control substance was placed on a 25 mm Hilltop Chamber® backed by adhesive tape (occlusive patch). The chambers were then applied to the clipped surface of the appropriate animals as quickly as possible.

Following chamber application, the trunk of the animal was wrapped with elastic wrap to prevent removal of the chamber and the animal was returned to its cage.

Approximately 6 hours after chamber application, the binding materials were removed. The test sites were then wiped 2 times with gauze moistened in mineral oil, followed by dry gauze, followed by gauze moistened in deionized water, followed by dry gauze, to remove test substance residue, and the animals were returned to their cages.

4.11.2.1. Induction

On the day prior to the first induction dose administration (Day -1), all main phase animals were weighed and the hair was removed from the left side of the test and HCA test animals. On the day following clipping (Day 0), chambers were applied as indicated in Text Table 4.

Text Table 4
Induction Dosing

Group	Test Material	Induction No.	Dose Volume (mL)	Dose Level Concentration (%)	Site	No. of Animals	
						Males	Females
Test	OS329036A	1	0.3	75[a]	1	10	10
		2	0.3	75[a]	1		
		3	0.3	75[a]	1[b]		
HCA Test	HCA	1	0.3	5.0[c]	1	5	5
		2	0.3	5.0[c]	1		
		3	0.3	5.0[c]	1[b]		

[a] The vehicle used was mineral oil.
[b] Test site was adjusted but remained at Site 1.
[c] The vehicle used was ethanol.

The induction procedure was repeated on Day 7 and Day 14 so that a total of 3 consecutive induction exposures were made to the test animals.

4.11.2.2. Challenge

On the day prior to challenge dose administration, the test, HCA test, challenge control, and HCA challenge control animals were weighed and the hair was removed from the right side of the animals. On the day following clipping (Day 28), chambers were applied as indicated in Text Table 5.

Text Table 5
Challenge Dosing

Group	Material	Dose Volume (mL)	Dose Level Concentration (%)	Site	No. of Animals	
					Males	Females
Test	OS329036A	0.3	35[a]	2	10	10
Challenge Control	OS329036A	0.3	35[a]	2	5	5
HCA Test	HCA	0.3	2.5[b]	2	5	5
		0.3	1.0[b]	4		
HCA Control	HCA	0.3	2.5[b]	2	5	5
		0.3	1.0[b]	4		

[a] The vehicle used was mineral oil.
[b] The vehicle used was acetone.

4.11.2.3. Rechallenge

On the day prior to rechallenge dose administration, the test and rechallenge control animals were weighed and the hair was removed from the right side of the animals. On the day following clipping (Day 35), chambers were applied as indicated in Text Table 6.

Text Table 6
Rechallenge Dosing

Group	Material	Concentration (%)	Site	No. of Animals	
				Males	Females
Test	OS329036A	15[a]	4	10	10
Challenge Control	OS329036A	15[a]	4	5	5

[a] The vehicle used was mineral oil.

4.12. In-life Procedures, Observations, and Measurements

The in-life procedures, observations, and measurements listed below were performed for main study animals.

4.12.1. Mortality/Moribundity Checks

The animals were observed for general health/mortality and moribundity twice daily, once in the morning and afternoon, throughout the study.

4.12.2. Clinical Observations

4.12.3. Detailed Clinical Observations

The animals were removed from the cage and examined in detail before dosing on Day 0.

4.12.4. Dermal Observations

The test sites of each main study animal were graded for irritation at approximately 24 hours and 48 hours after chamber application (induction) or 24 hours and 48 hours after chamber removal

(challenge and rechallenge) using the Macroscopic Dermal Grading System in Appendix 3 according to Buehler.[2]

4.12.5. Body Weights

Each main study animal was weighed on the day prior to the first induction (Day -1), on the day prior to challenge dosing for the appropriate test and challenge control animals, and on the day prior to rechallenge dosing for the appropriate test and rechallenge control animals.

4.12.6. Unscheduled Deaths

No unscheduled euthanasia occurred during the study. One HCA challenge control female, was found dead. This animal was subjected to a complete necropsy examination, which included evaluation of the carcass and musculoskeletal system; all external surfaces and orifices; cranial cavity and external surfaces of the brain; and thoracic, abdominal, and pelvic cavities with their associated organs and tissues. No tissues were retained.

4.12.7. Scheduled Euthanasia

Following the 48-hour scoring interval, all remaining main study animals were euthanized by carbon dioxide inhalation and discarded.

5. COMPUTERIZED SYSTEMS

Critical computerized systems used in the study are listed below. All computerized systems used in the conduct of this study have been validated; when a particular system has not satisfied all requirements, appropriate administrative and procedural controls were implemented to assure the quality and integrity of data.

Text Table 7
Critical Computerized Systems

System Name	Version No.	Description of Data Collected and/or Analyzed
Systems 600 Apogee Insight System	3.11	Temperature and/or humidity (animal rooms, refrigerators, freezers, and compound storage, as applicable)
Instem Life Science Systems, DISPENSE	8	Test material receipt, accountability and/or formulation activities

6. STATISTICAL ANALYSIS

The sensitization potential of the test substance was based on the dermal responses observed on the test and control animals at challenge and rechallenge. Generally, dermal scores of ≥ 1 in the test animals with scores of 0 to $\pm$ noted in the controls were considered indicative of sensitization. Dermal scores of 1 in both the test and control animals were generally considered equivocal unless a higher dermal response ($\geq$ grade 2) was noted in the test animals. Group mean dermal scores were calculated for challenge and rechallenge. A response of at least 15% in a nonadjuvant test was expected for a mild to moderate sensitizer in this study design.

7. RETENTION OF RECORDS, SAMPLES, AND SPECIMENS

All study-specific raw data, electronic data, documentation, protocol, retained samples and specimens, and final reports will be archived at the Testing Facility no later than the date of Final

Report issuance, and then transferred to the archive at Charles River Laboratories, Preclinical Services, Pennsylvania, Horsham, PA. Five years after issue of the audited draft report, the Sponsor will be contacted to determine the disposition of these materials.

Electronic data generated by the Testing Facility were archived as noted above, except that the data collected using Dispense 8 were archived at the Charles River Laboratories facility located in Wilmington, MA.

8. RESULTS

8.1. Mortality

No test substance-related mortality occurred during the study. Animal No. 6358 (HCA Challenge Control) was found dead on Day 28, 6 hours following dosing. This death was not considered to be HCA related, as mortality due to HCA administration has not been observed historically, and a specific cause of death was not identified at necropsy.

8.2. Range-Finding Phase

The Dermal Grading System is presented in Appendix 3.

(Table 1 and Table 2)

Exposure to OS329036A at concentrations of 50%, 75%, and 100% resulted in dermal scores of ±, 1, or 2. The 25% concentration resulted in dermal scores of 0 or ±. Therefore, induction was determined to be acceptable to 75%, as this was considered the highest concentration that resulted in acceptable dermal irritation that was considered tolerable throughout the induction phase. Since induction occurred at a concentration below 100%, the concentration for challenge or rechallenge would be required to be reduced. After exposure to OS329036A at concentrations of 5% and 15% resulted in dermal scores of 0, and concentrations of 25% and 35% resulted in dermal scores of 0 or ±, the challenge level of 35% and rechallenge level of 15% were selected.

8.3. Main Phase

The Dermal Grading System is presented in Appendix 3.

8.3.1. Induction Phase

(Table 3)

During the induction phase, dermal scores of ± (slight patchy erythema), 1 (slight, but confluent or moderate patchy erythema), and 2 (moderate, confluent erythema) were noted for the test animals. Additional observations included edema scores of 1 to 2, blanching, and desquamation.

8.3.2. Challenge Phase

(Table 4)

Following challenge with 35% OS329036A, dermal scores of 2 were noted in 18/20 test animals at the 24-hour scoring interval. At the 48-hour scoring interval, dermal scores of 2 were noted in 16/20 test animals. The remaining test animals had scores of 1 at the 24- and 48-hour scoring intervals. Dermal reactions in the challenge control animals were scores of 0, ±, or 1. Group mean dermal scores were higher in the test animals (1.8 to 1.9) as compared to challenge control animals (0.6).

Following challenge with 2.5% w/v HCA in acetone, dermal scores of 2 were noted in 10/10 HCA test animals at the 24-hour and 48-hour scoring intervals. Dermal reactions in the HCA control animals were limited to scores of 0. Group mean dermal scores were higher in the HCA test animals (2.0) compared to the HCA control animals (0.0).

Following challenge with 1.0% w/v HCA in acetone, dermal scores of 1 or 2 were noted in 10/10 HCA test animals at the 24-hour and 48-hour scoring intervals. Dermal reactions in the HCA control animals were limited to scores of 0. Group mean dermal scores were higher in the HCA test animals (1.7 to 1.8) compared to the HCA control animals (0.0).

8.3.3. Rechallenge Phase

(Table 5)

Following rechallenge with 15% OS329036A in mineral oil, dermal scores of 2 were noted in 14/20 test animals at the 24- and 48-hour scoring intervals. Dermal reactions in the remaining test animals were ± or 1. Dermal reactions in the rechallenge control animals were scores of 0, ±, or 1. Group mean dermal scores were higher in the test animals (1.7) as compared to rechallenge control animals (0.7 to 0.8).

8.4. Body Weights

(Appendix 4)

No OS329036A-related effects on body weight were observed in the test animals during the study. Weight gain in the animals throughout the study interval was indicative of good health in the test and control animals.

8.5. Clinical and Necropsy Observations

(Appendix 5)

No OS329036A-related clinical signs were observed during the study.

Animal No. 6358 (HCA Challenge Control female) was found dead on Day 28, at the time of binding removal. Necropsy results included dark material accumulation around nose, mouth, and right foot, along with lung discoloration and failure of the lungs to collapse.

9. CONCLUSION

Based on the results of this study, OS329036A is considered to be a contact sensitizer in guinea pigs, as the criterion for sensitization (dermal scores ≥ 2 in at least 15% of the test animals) was met. The results of the HCA positive control study demonstrated that a valid test was performed and indicated that the test design would detect potential contact sensitizers.

10. SCIENTIFIC REPORT REVIEW

This report has been reviewed for scientific content. The signature below indicates a concurrence with the Study Director's interpretation of these data as presented in this report.

_____ Date:_____

Mark A. Morse, PhD, DABT
Director of Research

11. REFERENCES

1. Guide for the care and use of laboratory animals. Washington, D.C.: National Academy Press. NRC (National Research Council); 2011.

2. Buehler EV. Delayed Contact Hypersensitivity in the Guinea Pig. Arch Dermat. 1965;91:171-177.

Table 1
Topical Range-Finding Data

STUDY NO. 20061359 PAGE 1

TABLE 1

A DERMAL SENSITIZATION STUDY IN GUINEA PIGS

TOPICAL RANGE-FINDING DATA
(0S329036A)

GROUP	ANIMAL NO./SEX BODY WEIGHT (G)	RANGE-FINDING DERMAL SCORES							
		100%[a]		75%[b]		50%[b]		25%[b]	
		24 HOURS	48 HOURS	24 HOURS	48 HOURS	24 HOURS	48 HOURS	24 HOURS	48 HOURS
RANGE-FINDING	6290/M (345)	2	1	1	±	1	0	0	0
	6291/M (334)	2	1	1	1	1	0	0	0
	6334/F (325)	1	±	1	1	1	0	0	0
	6335/F (331)	2	1	1	1	1	0	±	0

NOTE: SEE APPENDIX 3 FOR DEFINITION OF CODES.
[a]AS RECEIVED.
[b]THE VEHICLE USED WAS MINERAL OIL.

Table 2
Second Topical Range-Finding Data

STUDY NO. 20061359 PAGE 1

TABLE 2

A DERMAL SENSITIZATION STUDY IN GUINEA PIGS

SECOND TOPICAL RANGE-FINDING DATA
(OS329036A)

GROUP	ANIMAL NO./SEX BODY WEIGHT (G)	RANGE-FINDING DERMAL SCORES							
		35%[a]		25%[a]		15%[a]		5%[a]	
		24 HOURS	48 HOURS	24 HOURS	48 HOURS	24 HOURS	48 HOURS	24 HOURS	48 HOURS
RANGE-FINDING	6332/M (530)	±	0	±	0	0	0	0	0
	6333/M (556)	0	0	0	0	0	0	0	0
	6374/F (496)	0	0	0	0	0	0	0	0
	6375/F (502)	0	0	0	0	0	0	0	0

NOTE: SEE APPENDIX 3 FOR DEFINITION OF CODES.
[a]THE VEHICLE USED WAS MINERAL OIL.

Table 3
Individual Induction Data

STUDY NO. 20061359 PAGE 1

TABLE 3

A DERMAL SENSITIZATION STUDY IN GUINEA PIGS

INDIVIDUAL INDUCTION DATA
(OS329036A)

		DERMAL SCORES					
		INDUCTION I		INDUCTION II		INDUCTION III	
		75%[a]		75%[a]		75%[a, b]	
GROUP	ANIMAL NO./SEX	24 HOURS	48 HOURS	24 HOURS	48 HOURS	24 HOURS	48 HOURS
TEST	6292/M	1 ED-1	1	1 BLA-1, DES	1 BLA-1, DES	1 ED-1	2 BLA-1, ED-1,DES
	6293/M	1	±	2 BLA-1, ED-1	2 BLA-1, ED-1	2 ED-1	1 ED-1, DES
	6294/M	1 BLA-1, ED-1	1 BLA-1, ED-1	1	1 DES	2 ED-1	1 ED-1, DES
	6295/M	1 ED-1	1 ED-1	2 ED-1	2 ED-1, DES	2	2 DES
	6296/M	1	1	2 BLA-1	2 BLA-1, ED-1, DES	2	2 DES
	6297/M	1 ED-1	1 ED-1	1	1 BLA-1, ED-1	1 ED-1	1 BLA-1, ED-1, DES
	6298/M	2 ED-1	2 ED-1	2 BLA-1, ED-1	2 BLA-1, ED-1, DES	2 ED-1	2 BLA-1, ED-1, DES
	6299/M	1 ED-1	1 ED-1	2 BLA-1, ED-1	2 BLA-1, ED-2, DES	2 ED-1	2 BLA-1, ED-1, DES
	6300/M	1 ED-1	1 ED-1	2 BLA-1	2 BLA-1, ED-1, DES	1 ED-1	2 ED-1, DES
	6301/M	±	±	1 ED-1	2 ED-1, DES	2	2 ED-1, DES
	6336/F	1 ED-1	1 ED-1	1	2 ED-1	1 ED-1	2 BLA-1, ED-1, DES
	6337/F	1	1	2 BLA-1, ED-1	2 BLA-1, ED-1, DES	2 ED-1	2 ED-1, DES
	6339/F	±	±	2 BLA-1, ED-1	2 BLA-1, ED-2, DES	2 ED-1	2 ED-1, DES
	6340/F	1 BLA-1, ED-1	1 BLA-1, ED-1	2 ED-1	2 ED-1, DES	2 ED-1, BLA-1	2 BLA-1, ED-1, DES
	6341/F	1 ED-1	1 ED-1	2 ED-1	2 ED-1, DES	2 ED-1	2 ED-1, DES
	6342/F	2 ED-1	2 ED-1	1 ED-1	2 ED-1, DES	2 ED-1	2 ED-1, DES
	6343/F	± ED-1	± ED-1	2 BLA-1, ED-1	2 BLA-1, ED-2, DES	2 BLA-1, ED-2	2 BLA-1, ED-2, DES
	6344/F	1	±	1	1 DES	2 ED-1	2 BLA-1, ED-1, DES
	6345/F	1	1	2 BLA-1	2 BLA-1	1 BLA-1, ED-1	2 BLA-1, ED-2, DES
	6346/F	±	±	1 ED-1	2 ED-1	2 BLA-1, ED-1	2 BLA-1, ED-1

NOTE: SEE APPENDIX 3 FOR DEFINITION OF CODES.
[a]THE VEHICLE USED WAS MINERAL OIL.
[b]TEST SITE ADJUSTED BUT REMAINED AT SITE 1.

TABLE 3

A DERMAL SENSITIZATION STUDY IN GUINEA PIGS

INDIVIDUAL INDUCTION DATA
(HCA)

		DERMAL SCORES					
		INDUCTION I		INDUCTION II		INDUCTION III	
		5.0%[a]		5.0%[a]		5.0%[a, b]	
GROUP	ANIMAL NO./SEX	24 HOURS	48 HOURS	24 HOURS	48 HOURS	24 HOURS	48 HOURS
HCA TEST	6302/M	1	1	M-3^{BLA-2}	M-3$^{BLA-2, ED-1}$	2	2$^{BLA-1, DES}$
	6303/M	1	1	M-3$^{BLA-3, ES-1}$	M-3$^{BLA-3, ES-1}$	2^{BLA-1}	M-3$^{BLA-2, ED-1, DES}$
	6304/M	1	1	M-3^{BLA-3}	M-3$^{BLA-3, ED-1}$	2^{BLA-1}	M-3$^{BLA-2, DES}$
	6305/M	1	1	M-3$^{BLA-3, ES-1}$	M-3$^{BLA-3, ES-1, ED-1}$	1^{BLA-1}	2$^{BLA-1, ED-1}$
	6306/M	1	1	M-3^{BLA-3}	M-3$^{BLA-3, ED-1}$	M-3$^{BLA-3, ED-1}$	M-3$^{BLA-3, ED-1}$
	6347/F	1	1	M-3^{BLA-2}	M-3$^{BLA-2, ED-1}$	2^{BLA-1}	2$^{BLA-1, ED-1}$
	6348/F	1	1	M-3^{BLA-3}	M-3$^{BLA-2, ES-1, ED-1}$	1	2$^{BLA-1, ED-1, DES}$
	6349/F	1	1	M-3$^{BLA-2, ES-1}$	M-3$^{BLA-2, ES-1, ED-1}$	2	2$^{BLA-1, DES}$
	6350/F	1	1	M-3^{BLA-3}	M-3$^{BLA-2, ES-1, ED-1}$	M-3$^{BLA-3, ED-1}$	M-3$^{BLA-3, ED-1}$
	6351/F	1	1	M-3$^{BLA-3, ES-1}$	M-3$^{BLA-3, ES-1, ED-1}$	M-3$^{BLA-3, ED-1}$	M-3$^{BLA-3, ED-1}$

NOTE: SEE APPENDIX 3 FOR DEFINITION OF CODES.
[a]THE VEHICLE USED WAS ETHANOL.
[b]TEST SITE ADJUSTED BUT REMAINED AT SITE 1.

Table 4
Individual Challenge Data

STUDY NO. 20061359

TABLE 4

A DERMAL SENSITIZATION STUDY IN GUINEA PIGS

INDIVIDUAL CHALLENGE DATA
(OS319754)

		DERMAL SCORES	
		35%[a]	
GROUP	ANIMAL NO./SEX	24 HOURS	48 HOURS
TEST	6292/M	2	1
	6293/M	2	1
	6294/M	2	2
	6295/M	2	2
	6296/M	1	1
	6297/M	1	1
	6298/M	2^{ED-1}	2^{ED-1}
	6299/M	2	2
	6300/M	2^{ED-1}	2^{ED-1}
	6301/M	2^{ED-1}	2^{ED-1}
	6336/F	2	2
	6337/F	2^{ED-1}	2^{ED-1}
	6339/F	2	2
	6340/F	2	2
	6341/F	2	2
	6342/F	2	2
	6343/F	2	2
	6344/F	2	2
	6345/F	2	2
	6346/F	2	2
	MEAN	1.9	1.8

NOTE: SEE APPENDIX 3 FOR DEFINITION OF CODES.
[a]THE VEHICLE USED WAS MINERAL OIL.

TABLE 4

A DERMAL SENSITIZATION STUDY IN GUINEA PIGS

INDIVIDUAL CHALLENGE DATA
(OS329036A)

		DERMAL SCORES	
		35%[a]	
GROUP	ANIMAL NO./SEX	24 HOURS	48 HOURS
CHALLENGE	6307/M	±	±
CONTROL	6308/M	±	±
TEST	6309/M	±	±
	6310/M	±	±
	6312/M	1	1
	6352/F	0	0
	6353/F	1	1
	6354/F	±	±
	6355/F	0	0
	6356/F	1	1
	MEAN	0.6	0.6

NOTE: SEE APPENDIX 3 FOR DEFINITION OF CODES. FOR PURPOSES OF CALCULATION, ± = 0.5.
[a]VEHICLE USED WAS MINERAL OIL

TABLE 4

A DERMAL SENSITIZATION STUDY IN GUINEA PIGS

INDIVIDUAL CHALLENGE DATA
(OS329036A)

		DERMAL SCORES			
		2.5%[a]		1.0[a]	
GROUP	ANIMAL NO./SEX	24 HOURS	48 HOURS	24 HOURS	48 HOURS
HCA TEST	6302/M	2	2	2	2
	6303/M	2	2	1	2
	6304/M	2	2	1	1
	6305/M	2	2	1	1
	6306/M	2	2	2	2
	6347/F	2	2	2	2
	6348/F	2	2	2	2
	6349/F	2	2	2	2
	6350/F	2	2	2	2
	6351/F	2	2	2	2
	MEAN	2.0	2.0	1.7	1.8

NOTE: SEE PROTOCOL ATTACHMENT 1 FOR DEFINITION OF CODES.
[a]THE VEHICLE USED WAS ACETONE.

STUDY NO. 20061359

TABLE 4

A DERMAL SENSITIZATION STUDY IN GUINEA PIGS

INDIVIDUAL CHALLENGE DATA
(0S329036A)

		DERMAL SCORES			
		2.5%[a]		1.0[a]	
GROUP	ANIMAL NO./SEX	24 HOURS	48 HOURS	24 HOURS	48 HOURS
HCA	6313/M	0	0	0	0
CHALLENGE	6314/M	0	0	0	0
CONTROL	6315/M	0	0	0	0
	6316/M	0	0	0	0
	6317/M	0	0	0	0
	6357/F	0	0	0	0
	6358/F[b]	-	-	-	-
	6359/F	0	0	0	0
	6360/F	0	0	0	0
	6361/F	0	0	0	0
	MEAN	0.0	0.0	0.0	0.0

NOTE: SEE PROTOCOL ATTACHMENT 1 FOR DEFINITION OF CODES.
[a]THE VEHICLE USED WAS ACETONE.
[b]ANIMAL WAS FOUND DEAD AT TIME OF RINSING. ANIMAL RINSED PRIOR TO NECROPSY SUBMISSION.

Table 5
Individual Rechallenge Data

TABLE 5

A DERMAL SENSITIZATION STUDY IN GUINEA PIGS

INDIVIDUAL RECHALLENGE DATA
(0S329036A)

		DERMAL SCORES	
		15%[a]	
GROUP	ANIMAL NO./SEX	24 HOURS	48 HOURS
TEST	6292/M	2	2
	6293/M	2	2
	6294/M	2	2
	6295/M	2	2
	6296/M	2	2
	6297/M	2	2
	6298/M	2	2
	6299/M	2	2
	6300/M	2	2
	6301/M	2	2
	6336/F	2	2
	6337/F	2	2
	6339/F	1	1
	6340/F	2	2
	6341/F	2	2
	6342/F	1	1
	6343/F	±	±
	6344/F	1	1
	6345/F	1	1
	6346/F	1	1
	MEAN	1.7	1.7

NOTE: SEE APPENDIX 3 FOR DEFINITION OF CODES. FOR PURPOSES OF CALCULATION, ± = 0.5.
[a]THE VEHICLE USED WAS MINERAL OIL.

TABLE 5

A DERMAL SENSITIZATION STUDY IN GUINEA PIGS

INDIVIDUAL RECHALLENGE DATA
(0S329036A)

		DERMAL SCORES	
		15%[a]	
GROUP	ANIMAL NO./SEX	24 HOURS	48 HOURS
RECHALLENGE	6318/M	1	1
CONTROL	6320/M	±	1
	6321/M	1	1
	6322/M	±	1
	6323/M	1	1
	6362/F	±	±
	6363/F	±	±
	6365/F	±	±
	6366/F	1	1
	6367/F	0	±
	MEAN	0.7	0.8

NOTE: SEE APPENDIX 3 FOR DEFINITION OF CODES. FOR PURPOSES OF CALCULATION, ± = 0.5.
[a]THE VEHICLE USED WAS MINERAL OIL.

Appendix 1
Protocol and Protocol Amendment

charles river

FINAL PROTOCOL

Testing Facility Study No. 20061359

A Sensitization Study of OS329036A by Dermal Administration in Guinea Pigs-Modified Buehler Design

SPONSOR:
The Lubrizol Corporation
29400 Lakeland Blvd.
Wickliffe, OH 44092-2298
United States

TESTING FACILITY:
Charles River Laboratories
Preclinical Services, Ohio (PCS-OH)
640 North Elizabeth Street
Spencerville, OH 45887
United States

15 September 2014

Page 1 of 23

TABLE OF CONTENTS

1. OBJECTIVE(S) 3
2. PROPOSED STUDY SCHEDULE 3
3. GUIDELINES FOR STUDY DESIGN 3
4. REGULATORY COMPLIANCE 4
5. QUALITY ASSURANCE 4
6. SPONSOR 4
7. RESPONSIBLE PERSONNEL 5
8. TEST AND CONTROL SUBSTANCES 5
9. SAFETY 7
10. DOSE FORMULATION 8
11. TEST SYSTEM 8
12. HUSBANDRY 9
13. EXPERIMENTAL DESIGN 11
14. IN-LIFE PROCEDURES, OBSERVATIONS, AND MEASUREMENTS-RANGE-FINDING STUDY 15
15. TERMINAL PROCEDURES-RANGE-FINDING STUDY 15
16. IN-LIFE PROCEDURES, OBSERVATIONS, AND MEASUREMENTS-MAIN STUDY 16
17. TERMINAL PROCEDURES-MAIN STUDY 17
18. COMPUTERIZED SYSTEMS 19
19. STATISTICAL ANALYSIS 19
20. AMENDMENTS AND DEVIATIONS 19
21. RETENTION OF RECORDS, SAMPLES, AND SPECIMENS 20
22. REPORTING 20
23. ANIMAL WELFARE 20
24. REFERENCES 21
25. TESTING FACILITY APPROVAL 22
26. SPONSOR APPROVAL 23

1. OBJECTIVE(S)

The objective of this study is to assess the dermal sensitization potential of OS329036A when given as multiple topical applications to guinea pigs.

1.1. SEND Study Classification

Study Category:	Toxicology
Study Type:	Repeat Dose Toxicity
Study Design:	Parallel
Primary Treatment CAS Registry Number:	Not Available
Primary Treatment Unique Ingredient ID:	Not Available
Class of Compound:	Not Available

2. PROPOSED STUDY SCHEDULE

Proposed study dates are listed below. Actual applicable dates will be included in the Final Report.

Experimental Start Date (OECD):	23 Sep 2014 (First date of study-specific data collection)
Experimental Start Date (EPA):	30 Sep 2014 (First date test substance is applied to the test system)
Experimental Completion Date (OECD):	6 months following issuance of the Draft Report
Experimental Termination Date (EPA):	To be included in the Final Report
Animal Arrival/Transfer:	23 Sep 2014
Initiation of Dosing:	30 Sep 2014
Completion of In-life:	To be included in the Final Report
Audited Draft Report:	6 weeks following completion of in-life

3. GUIDELINES FOR STUDY DESIGN

The design of this study was based on the study objective(s), the overall product development strategy for the test substance, and the following study design guidelines:

- OECD Guideline 406. *Skin Sensitisation.*
- EPA Health Effects Test Guideline OPPTS 870.2600: *Skin Sensitization.*

4. REGULATORY COMPLIANCE

The study will be performed in accordance with the United States Code of Federal Regulations, Title 40, Part 792: Good Laboratory Practice Standards and as accepted by Regulatory Authorities throughout the European Union (OECD Principles of Good Laboratory Practice), Japan (MAFF and METI), and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

Exceptions to GLPs include the following study elements:

- Characterization of the test substance was performed by the Sponsor or Sponsor subcontractor according to established SOPs, controls, and approved test methodologies to ensure integrity and validity of the results generated; these analyses were not conducted in compliance with the GLP or GMP regulations.
- Concentration, stability, and homogeneity of the test substance formulations will not be determined in this study.
- Concentration, stability and homogeneity of the α-Hexylcinnamaldehyde (HCA) formulations will not be determined in this study.

5. QUALITY ASSURANCE

5.1. Testing Facility

The Testing Facility Quality Assurance Unit (QAU) will monitor the study to assure the facilities, equipment, personnel, methods, practices, records, and controls are in conformance with Good Laboratory Practice regulations. The QAU will review the protocol, conduct inspections at intervals adequate to assure the integrity of the study, and audit the Final Report to assure that it accurately describes the methods and standard operating procedures and the reported results accurately reflect the raw data of the study.

6. SPONSOR

Sponsor Representative

Robert Hinderer, PhD
Tel: 440.347.5181
E-mail: robert.hinderer@lubrizol.com

7. RESPONSIBLE PERSONNEL

Study Director

Jason W. Smedley, BS
Address as cited for Testing Facility
Tel: 419.647.4196
Fax: 419.647.6560
E-mail: jason.smedley@crl.com

Management Contact

Mark A. Morse, PhD, DABT
Tel: 419.647.4196
Fax: 419.647.6560
E-mail: mark.morse@crl.com

8. TEST AND CONTROL SUBSTANCES

8.1. Test Substance(s)

Identification: OS329036A

Batch (Lot) Number: OS329036A

Expiration Date: To be included in the Final Report

Physical Description: Liquid

Correction Factors:

Name	Base/Salt Conversion	Purity	Hygroscopic Water	Total Correction (base/salt×purity×hygroscopic water)
OS329036A	N/A	100%[a]	N/A	100%
[a] Dose calculations will not be corrected for purity.				

Storage Conditions: Kept in a controlled room temperature area

8.2. Control Substance(s)

Identification: Mineral Oil

Supplier: To be included in the Final Report

Batch (Lot) Number: To be included in the Final Report

Expiration Date: To be included in the Final Report

Physical Description: Liquid

Storage Conditions: Kept in a room temperature area

8.3. Positive Control

Identification: 5.0% (w/v) α-Hexylcinnamaldehyde in Ethanol

Identification: 2.5% (w/v) α-Hexylcinnamaldehyde in Acetone

Identification: 1.0% (w/v) α-Hexylcinnamaldehyde in Acetone

8.3.1. Positive Control Substance Components

Identification: α-Hexylcinnamaldehyde

Supplier: To be included in the Final Report

Batch (Lot) Number: To be included in the Final Report

Expiration Date: To be included in the Final Report

Physical Description: Liquid

Storage Conditions: Kept in a room temperature area, protect from light, desiccate

Identification: Acetone

Supplier: To be included in the Final Report

Batch (Lot) Number: To be included in the Final Report

Expiration Date: To be included in the Final Report

Physical Description: Liquid

Storage Conditions: Kept in a room temperature area

Identification: Ethanol

Supplier: To be included in the Final Report

Batch (Lot) Number: To be included in the Final Report

Expiration Date: To be included in the Final Report

Physical Description: Liquid

Storage Conditions: Kept in a room temperature area

8.4. Test Substance Characterization

The Sponsor will provide to the Testing Facility documentation of the identity, strength, purity, composition, and stability for the test substance. A Certificate of Analysis or equivalent

documentation will be provided for inclusion in the Final Report. The Sponsor will also provide information concerning the regulatory standard that was followed for these evaluations.

The Sponsor has appropriate documentation on file concerning the method of synthesis, fabrication or derivation of the test substance, and this information is available to the appropriate regulatory agencies should it be requested.

8.5. Analysis of Test Substance

The stability of the bulk test substance will not be determined during the course of this study. Information to support the stability of each lot of the bulk test substance will be provided by the Sponsor.

8.6. Reserve Samples

For each batch (lot) of test and control substance, a reserve sample (1 g/1 mL) will be collected and maintained under the appropriate storage conditions by the Testing Facility if the experimental period of the study is 4 weeks or longer.

8.7. Test Substance Inventory and Disposition

Records of the receipt, distribution, storage, and disposition of test substance (including empty containers) will be maintained. With the exception of reserve samples, all unused Sponsor-supplied bulk test substance will be returned to the Sponsor (after issue of the Final Reports of all studies using these materials, unless otherwise instructed by the Sponsor). All empty containers will be maintained for the duration of the study.

Shipping Contact
Tina Adams
The Lubrizol Corporation
29400 Lakeland Blvd.
Wickliffe, OH 44092-2298
Tel: 440.347.8509
E-mail: tina.adams@lubrizol.com

9. SAFETY

The following safety instructions apply to this study:

Standard laboratory safety procedures will be employed for handling the test and control substance(s). Specifically, laboratory gloves, laboratory coat, and eye protection will be worn. Safety information on the test substance will be provided by the Sponsor in the form of a Material Safety Data Sheet or equivalent, if available.

10. DOSE FORMULATION

10.1. Preparation of Test Substance

The test substance, OS329036A, will be administered as received and/or diluted with the control substance on the day of dosing. Selected doses will be achieved by adjustment of test substance concentration in the control substance.

Any residual volumes will be discarded unless otherwise requested by the Study Director.

10.2. Preparation of α-Hexylcinnamaldehyde (HCA) Positive Control

The α-Hexylcinnamaldehyde will be administered at a 5.0% w/v concentration in an ethanol vehicle for induction and at 2.5% w/v and 1.0% w/v concentrations in the acetone vehicle for challenge. The α-Hexylcinnamaldehyde solution for induction and challenge will be prepared as follows: 0.50 g of α-Hexylcinnamaldehyde (induction) or 0.25 g and 0.10 g of α-Hexylcinnamaldehyde (challenge) will each be brought to a volume of 10 mL with the appropriate solvent to produce the required concentrations. The α-Hexylcinnamaldehyde will be prepared, protected from light, and dispensed on the day of dosing.

Any residual volumes will be discarded unless otherwise requested by the Study Director.

10.3. Sample Collection and Analysis

No samples for analytical analysis will be collected by the Testing Facility.

11. TEST SYSTEM

Species:	Guinea pig
Strain:	Hartley-derived albino guinea pig
Source:	Charles River Laboratories, Kingston, NY
Number of Males Ordered:	44
Number of Females Ordered:	44
Target Age at the Initiation of Dosing:	Young adults
Target Weight at the Initiation of Dosing:	300 to 500 g

The actual age, weight, and number of animals received will be listed in the Final Report.

11.1. Justification of Test System and Number of Animals

The Hartley-derived guinea pig was chosen as the animal model for this study as it is an accepted rodent species for preclinical toxicity testing by regulatory agencies.

The total number of animals to be used in this study is considered to be the minimum required to properly characterize the effects of the test substance. This study has been designed such that it does not require an unnecessary number of animals to accomplish its objectives.

At this time, studies in laboratory animals provide the best available basis for extrapolation to humans and are required to support regulatory submissions. Acceptable models which do not use live animals currently do not exist.

11.2. Animal Identification

Each animal will be identified using a plastic ear tag.

11.3. Environmental Acclimation

The animals will be acclimated to their designated housing for at least 5 days before the first day of dosing.

11.4. Selection, Assignment, Replacement, and Disposition of Animals

The animals chosen for study will be arbitrarily selected from healthy stock animals. Animals in poor health will not be assigned to groups.

The disposition of all animals will be documented in the study records.

12. HUSBANDRY

12.1. Housing

The animals will be group housed (2 animals of the same sex and same dosing group together) in polycarbonate cages containing appropriate bedding equipped with an automatic watering valve as specified in the USDA Animal Welfare Act (9 CFR, Parts 1, 2 and 3) and as described in the *Guide for the Care and Use of Laboratory Animals*.[1] As an alternative, guinea pigs may be individually housed in solid bottom cages containing a hiding device and direct bedding material. These housing conditions will be maintained unless deemed inappropriate by the Study Director and/or Clinical Veterinarian. The room(s) in which the animals will be kept will be documented in the study records.

Animals will be separated during designated procedures/activities. Each cage will be clearly labeled with a color-coded cage card indicating study, group, animal number(s), and sex. Cages will be arranged on the racks in group order. Where possible, control group animals will be housed on a separate rack from the test substance treated animals.

12.2. Environmental Conditions

The targeted conditions for animal room environment will be as follows:

Temperature:	68°F to 79°F (20°C to 26°C)
Humidity:	30% to 70%
Light Cycle:	12 hours light and 12 hours dark (except during designated procedures)
Ventilation:	10 or more air changes per hour

12.3. Food

PMI Nutrition International Certified Guinea Pig Chow No. 5026 will be provided ad libitum throughout the study, except during designated procedures. The same diet in meal form may be provided to individual animals as warranted by clinical signs (e.g., broken/damaged incisors or other health changes).

The feed is analyzed by the supplier for nutritional components and environmental contaminants. Results of the analysis are provided by the supplier and are on file at the Testing Facility.

It is considered that there are no known contaminants in the feed that would interfere with the objectives of the study.

12.4. Water

Municipal tap water after treatment by reverse osmosis and ultraviolet irradiation will be freely available to each animal via an automatic watering system (except during designated procedures). Water bottles and/or supplemental water gel can be provided, if required.

Periodic analysis of the water is performed, and results of these analyses are on file at the Testing Facility.

It is considered that there are no known contaminants in the water that could interfere with the outcome of the study.

12.5. Animal Enrichment

Beginning at receipt, guinea pigs will be pair housed in solid bottom cages containing direct bedding material. As an alternative, guinea pigs may be individually housed in solid bottom cages containing direct bedding material. When individually housed, a hiding comfort device (PVC pipe) may be provided. In addition, the animals will receive a certified timothy hay cube at least weekly.

12.6. Veterinary Care

Veterinary care will be available throughout the course of the study and animals will be examined by the veterinary staff as warranted by clinical signs or other changes. All veterinary examinations and recommended therapeutic treatments, if any, will be documented in the study records.

In the event that animals show signs of illness or distress, the responsible veterinarian may make initial recommendations about treatment of the animal(s) and/or alteration of study procedures, which must be approved by the Study Director. All such actions will be properly documented in the study records and, when appropriate, by protocol amendment. Treatment of the animal(s) for minor injuries or ailments may be approved without prior consultation with the Sponsor representative when such treatment does not impact fulfillment of the study objectives. If the condition of the animal(s) warrants significant therapeutic intervention or alterations in study procedures, the Sponsor representative will be contacted, when possible, to discuss appropriate action. If the condition of the animal(s) is such that emergency measures must be taken, the Study Director and/or attending veterinarian will attempt to consult with the Sponsor representative prior to responding to the medical crisis, but the Study Director and/or veterinarian has authority to act immediately at his/her discretion to alleviate suffering. The Sponsor representative will be fully informed of any such events.

13. EXPERIMENTAL DESIGN

Experimental Design-Range-Finding Study

Site No.	Test Material	Dose Level	Number of Animals[a]	
			Males	Females
1	OS329036A	100%	2	2
2	OS329036A	75%		
3	OS329036A	50%		
4	OS329036A	25%		

[a] Additional animals may be necessary depending on the results obtained at each level.

Experimental Design-Main Study

Group	Phase/Treatment				Number of Animals[a]	
	Induction 1 to 3	Challenge	Rechallenge[a]	Second Rechallenge[b]	Males	Females
Test	Test Substance	Test Substance	Test Substance	Test Substance	10	10
Challenge Control	-	Test Substance	-	-	5	5
Rechallenge Control	-	-	Test Substance	-	5	5
Second Rechallenge Control	-	-	-	Test Substance	5	5
HCA Test	5.0% HCA	2.5% and 1.0% HCA	-	-	5	5
HCA Control	-	2.5% and 1.0% HCA	-	-	5	5

- = not applicable.
[a] To be conducted only if needed to clarify the primary challenge results.
[b] To be conducted only if needed to clarify the rechallenge results.

13.1. Administration of Test and Control Substances

Range-Finding Study: On the day prior to dose administration, the hair will be removed from the right and left sides of four guinea pigs with a small animal clipper. Care will be taken to avoid abrading the skin during clipping procedures.

On the following day, up to four closed chambers at four different concentrations of test substance will be applied to the clipped area of each animal (one 25-mm chamber for each level of test substance). A dose of 0.3 mL (or maximum volume for viscous materials) will be placed on a 25-mm Hill Top Chamber® backed by adhesive tape (occlusive patch). The chambers will then be applied to the clipped surface as quickly as possible. The trunk of the animal will be wrapped with elastic wrap which is secured with adhesive tape (if necessary) to prevent removal of the chamber. Six hours after chamber application, the elastic wrap, tape, and chambers will be removed. The test sites will then be wiped 2 times with gauze moistened in mineral oil, followed by dry gauze and then be wiped with gauze moistened in deionized water, followed by dry gauze, to remove test substance residue. If the mineral oil followed by deionized water does not sufficiently remove the test substance residue, the Study Director/Sponsor may choose to use another solvent.

Main Study: For the induction, challenge, and rechallenge phases, a dose of 0.3 mL (or maximum volume for viscous materials) will be placed on a 25-mm Hill Top Chamber® backed by adhesive tape (occlusive patch). The chambers will then be applied to the clipped surface as quickly as possible.

Main Study Induction: On the day prior to the first induction dose administration (Day -1), the hair will be removed from the left side of the test animals with a small animal clipper. Care will be taken to avoid abrading the skin during the clipping procedures. On the day following clipping (Day 0), chambers containing the appropriate material will be applied to the clipped area of the test animals and α-Hexylcinnamaldehyde test animals. The induction procedure will be repeated on Day 7 (±1 day) and Day 14 (±1 day) so that a total of three consecutive induction exposures will be made to the test animals and α-Hexylcinnamaldehyde test animals. The application site for induction may be moved if irritation persists from a previous induction exposure (to ensure the test substance is not dosed on compromised skin) but will remain on the left side of the animal. Following chamber application, the trunk of the animal will be wrapped with elastic wrap which is secured with adhesive tape (if necessary) to prevent removal of the chamber.

Six hours after chamber application, the elastic wrap, tape, and chambers will be removed. The test sites will then be wiped 2 times with gauze moistened in mineral oil, followed by dry gauze and then be wiped with gauze moistened in deionized water, followed by dry gauze, to remove test substance residue. If the mineral oil followed by deionized water does not sufficiently remove the test substance residue, the Study Director/Sponsor may choose to use another solvent.

Main Study Challenge: On the day prior to challenge dose administration, the hair will be removed from the right side of the test and challenge control animals and α-Hexylcinnamaldehyde test and control animals. Care will be taken to avoid abrading the skin during the clipping procedures. On the day following clipping (Day 28 ± 1 day), chambers containing the appropriate material will be applied to a naive site within the clipped area of the test and challenge control animals and α-Hexylcinnamaldehyde test and control animals. Following chamber application, the trunk of the animal will be wrapped with elastic wrap which is secured with adhesive tape (if necessary) to prevent removal of the chamber.

Six hours after chamber application, the elastic wrap, tape, and chambers will be removed. The test sites will then be wiped 2 times with gauze moistened in mineral oil, followed by dry gauze and then be wiped with gauze moistened in deionized water, followed by dry gauze, to remove test substance residue. If the mineral oil followed by deionized water does not sufficiently remove the test substance residue, the Study Director/Sponsor may choose to use another solvent.

Main Study Rechallenge: On the day prior to rechallenge dose administration, the hair will be removed from the right side of the test and rechallenge control animals. Care will be taken to avoid abrading the skin during the clipping procedures. On the day following clipping (Day 35 ± 1 day), chambers containing the test substance will be applied to a naive site within the clipped area of the test and rechallenge control animals. Following chamber application, the trunk of the animal will be wrapped with elastic wrap which is secured with adhesive tape (if necessary) to prevent removal of the chamber.

Six hours after chamber application, the elastic wrap, tape, and chambers will be removed. The test sites will then be wiped 2 times with gauze moistened in mineral oil, followed by dry gauze and then be wiped with gauze moistened in deionized water, followed by dry gauze, to remove test substance residue. If the mineral oil followed by deionized water does not sufficiently remove the test substance residue, the Study Director/Sponsor may choose to use another solvent.

Main Phase Second-Rechallenge: On the day prior to second-rechallenge dose administration, the hair will be removed from the right side of the test and second-rechallenge control animals. Care will be taken to avoid abrading the skin during the clipping procedures. On the day following clipping (Day 42 ± 1 day), chambers containing the test article will be applied to a naive site within the clipped area of the test and second-rechallenge control animals. Following chamber application, the trunk of the animal will be wrapped with elastic wrap which is secured with adhesive tape (if necessary) to prevent removal of the chamber.

Six hours after chamber application, the elastic wrap, tape, and chambers will be removed. The test sites will then be wiped 2 times with gauze moistened in mineral oil, followed by dry gauze and then be wiped with gauze moistened in deionized water, followed by dry gauze, to remove test substance residue. If the mineral oil followed by deionized water does not sufficiently remove the test substance residue, the Study Director/Sponsor may choose to use another solvent.

13.2. Justification of Route and Dose Levels

The dermal route of exposure was selected because this is the intended route of human exposure.

Range-Finding Study: Four graded levels are utilized for this procedure. Optimally, the range-finding study should produce no systemic toxicity and a spectrum of dermal responses that include Grades 0, ±, 1, and 2 unless the test substance is not dermally irritating at 100%.

Main Study: Optimally, the test substance concentration used for induction should produce no systemic toxicity and a mild to moderate dermal response (Grades ±, 1, or 2) unless the test substance is not dermally irritating at 100%. The test substance concentration may be varied during the induction period depending on the dermal responses produced. The test substance concentration(s) used for challenge should produce no systemic toxicity and dermal responses generally consist of Grades 0 to ± unless the test substance is not dermally irritating at 100%.

If the results of the challenge procedure are not conclusive, then a rechallenge may need to be performed to help clarify the challenge responses. The test substance concentration(s) used for rechallenge should produce no systemic toxicity and dermal responses generally consisting of Grades 0 to ± unless the test substance is not dermally irritating at 100%. The dose concentration for the main study was based upon the results of the range-finding portion of the study.

14. IN-LIFE PROCEDURES, OBSERVATIONS, AND MEASUREMENTS-RANGE-FINDING STUDY

14.1. Mortality/Moribundity Checks

Frequency: Twice daily, once in the morning and once in the afternoon, throughout the study.

Procedure: Animals will be observed for general health/mortality and moribundity. Animals will not be removed from cage during observation, unless necessary for identification or confirmation of possible findings.

14.2. Clinical Observations

14.2.1. Detailed Clinical Observations

Frequency: Day 0 (before dosing).

Procedure: Animals removed from the cage for examination.

14.3. Dermal Scoring

Frequency: 24 and 48 hours after chamber application.

Procedure: Each animal will be removed from the cage and test sites will be graded for irritation according to Buehler.[2] An alternative light source may be used to aid in dermal scoring.

14.4. Body Weights

Frequency: Day -1.

Procedure: Animals will be individually weighed.

15. TERMINAL PROCEDURES-RANGE-FINDING STUDY

Terminal procedures are summarized in the following table:

Terminal Procedures for Range-Finding Animals

Number of Animals		Scheduled Euthanasia Day	Necropsy Procedures	
M	F		Necropsy	Tissue Collection
2	2	2	-	-
Unscheduled Deaths			X	-

X = procedure to be conducted; - = not applicable.

15.1. Unscheduled Deaths

If a range-finding study animal dies on study, a necropsy will be conducted. If necessary, the animal will be refrigerated to minimize autolysis.

Range-finding study animals may be euthanized for humane reasons as per Testing Facility SOPs. These animals will undergo necropsy. If necessary, the animal will be refrigerated to minimize autolysis.

15.2. Scheduled Euthanasia

Range-finding study animals surviving until scheduled euthanasia will be euthanized by carbon dioxide inhalation and discarded.

15.3. Necropsy

All range-finding study animals found dead or euthanized moribund will be subjected to a complete necropsy examination, which will include evaluation of the carcass and musculoskeletal system; all external surfaces and orifices; cranial cavity and external surfaces of the brain; and thoracic, abdominal, and pelvic cavities with their associated organs and tissues. No tissues will be retained.

Necropsy procedures will be performed by qualified personnel with appropriate training and experience in animal anatomy and gross pathology.

Images may be generated for illustration of or consultation on gross observations. Generation of such images will be documented. Images and associated documentation will be retained and archived.

16. IN-LIFE PROCEDURES, OBSERVATIONS, AND MEASUREMENTS-MAIN STUDY

16.1. Mortality/Moribundity Checks

Frequency:	Twice daily, once in the morning and once in the afternoon, throughout the study.
Procedure:	Animals will be observed for general health/mortality and moribundity. Animals will not be removed from cage during observation, unless necessary for identification or confirmation of possible findings.

16.2. Clinical Observations

16.2.1. Detailed Clinical Observations

Frequency:	Day 0 (before dosing).
Procedure:	Animals removed from the cage for examination.

16.3. Dermal Scoring

Frequency:	24 and 48 hours after chamber application at inductions and 24 and 48 hours after chamber removal at challenge, rechallenge, and second rechallenge. A 72-hour grade may be conducted as deemed necessary by the Study Director/Sponsor to allow further evaluation of challenge responses.
Procedure:	Each animal will be removed from the cage and test sites will be graded for irritation according to Buehler.[2] An alternative light source may be used to aid in dermal scoring.

16.4. Body Weights

Frequency:	Day -1 (prior to first induction), the day prior to challenge dosing, the day prior to rechallenge dosing, and the day prior to second-rechallenge dosing.
Procedure:	All animals will be individually weighed prior to first induction. The test, HCA test, challenge control and HCA control animals will be individually weights prior to challenge dosing. The test and rechallenge animals will be individually weighed prior to rechallenge, and the test and second rechallenge animals will be individually weighed prior to second rechallenge.

17. TERMINAL PROCEDURES-MAIN STUDY

Terminal procedures are summarized in the following table:

Terminal Procedures for Main Animals

Group	Number of Animals		Scheduled Euthanasia Day	Necropsy Procedures	
	M	F		Necropsy	Tissue Collection
Test	10	10	[a]	-	-
Challenge	5	5	[a]	-	-
Rechallenge	5	5	[a]	-	-
Second Rechallenge	5	5	[a]	-	-
HCA Test	5	5	[a]	-	-
HCA Control	5	5	[a]	-	-
Unscheduled Deaths				X	-

X = procedure to be conducted; - = not applicable.
[a] Animals euthanized upon authorization from the Study Director.

17.1. Unscheduled Deaths

If a main study animal dies on study, a necropsy will be conducted. If necessary, the animal will be refrigerated to minimize autolysis.

Main study animals may be euthanized for humane reasons as per Testing Facility SOPs. These animals will undergo necropsy. If necessary, the animal will be refrigerated to minimize autolysis.

17.2. Scheduled Euthanasia

Main study animals surviving until scheduled euthanasia will be euthanized by carbon dioxide inhalation and discarded.

17.3. Necropsy

All main study animals found dead or euthanized moribund will be subjected to a complete necropsy examination, which will include evaluation of the carcass and musculoskeletal system; all external surfaces and orifices; cranial cavity and external surfaces of the brain; and thoracic, abdominal, and pelvic cavities with their associated organs and tissues. No tissues will be retained.

Necropsy procedures will be performed by qualified personnel with appropriate training and experience in animal anatomy and gross pathology.

Images may be generated for illustration of or consultation on gross observations. Generation of such images will be documented. Images and associated documentation will be retained and archived.

18. COMPUTERIZED SYSTEMS

The following critical computerized systems may be used in the study. The actual critical computerized systems used will be specified in the Final Report.

Data for parameters not required by protocol, which are automatically generated by analytical devices used will be retained on file but not reported. Statistical analysis results that are generated by the program but are not required by protocol and/or are not scientifically relevant will be retained on file but will not be included in the tabulations.

Critical Computerized Systems

System Name	Description of Data Collected and/or Analyzed
Compaq Alpha DS10 Computer using the Toxicology Analysis System Customized, Acute Toxicology Module or Provantis	applicable in-life data
Systems 600 Apogee Insight System	temperature and/or humidity (animal rooms, refrigerators, freezers, and compound storage)
Instem Life Science Systems, DISPENSE	test material receipt, accountability and/or formulation activities

19. STATISTICAL ANALYSIS

The sensitization potential of the test substance will be based on the dermal responses observed on the test and control animals at challenge and rechallenge (if conducted). Generally, dermal scores of ≥1 in the test animals with scores of 0 to ± noted in the controls are considered indicative of sensitization. A dermal score of 1 in both the test and control animals is generally considered equivocal unless a higher dermal response (≥ Grade 2) is noted in the test animals. Group mean dermal scores will be calculated for challenge and rechallenge (if conducted). A response of at least 15% in a nonadjuvant test should be expected for a mild to moderate sensitizer.

20. AMENDMENTS AND DEVIATIONS

Changes to the approved protocol shall be made in the form of an amendment, which will be signed and dated by the Study Director. Every reasonable effort will be made to discuss any necessary protocol changes in advance with the Sponsor.

All protocol and SOP deviations will be documented in the study records. Deviations from the protocol and/or SOP related to the phase(s) of the study conducted at a Test Site shall be documented, acknowledged by the PI/IS, and reported to the Study Director for authorization/acknowledgement. The Study Director will notify the Sponsor of deviations that may result in a significant impact on the study as soon as possible.

21. RETENTION OF RECORDS, SAMPLES, AND SPECIMENS

All study-specific raw data, electronic data, documentation, protocol, retained samples and specimens, and interim (if applicable) and final reports from this study will be transferred to a Charles River archive by no later than the date of final report issue. Five years after issue of the audited draft report, the Sponsor will be contacted to determine the disposition of materials associated with the study.

22. REPORTING

A comprehensive Draft Report will be prepared following completion of the study and will be finalized following consultation with the Sponsor. The report will include all information necessary to provide a complete and accurate description of the experimental methods and results and any circumstances that may have affected the quality or integrity of the study.

The Sponsor will receive an electronic version of the Draft and Final Report provided in Adobe Acrobat PDF format (hyperlinked and searchable at final) along with a Microsoft Word version of the text. The PDF document will be created from native electronic files to the extent possible, including text and tables generated by the Testing Facility. Report components not available in native electronic files and/or original signature pages will be scanned and converted to PDF image files for incorporation. An original copy of the report with the Testing Facility's handwritten signatures will be retained.

Reports should be finalized within 6 months of issue of the audited Draft Report. If the Sponsor has not provided comments to the report within 6 months of draft issue, the report will be finalized by the Testing Facility unless other arrangements are made by the Sponsor.

23. ANIMAL WELFARE

This study will comply with all applicable sections of the Final Rules of the Animal Welfare Act regulations (Code of Federal Regulations, Title 9), the *Public Health Service Policy on Humane Care and Use of Laboratory Animals* from the Office of Laboratory Animal Welfare, and the *Guide for the Care and Use of Laboratory Animals* from the National Research Council.[1,3] The protocol and any amendments or procedures involving the care or use of animals in this study will be reviewed and approved by the Testing Facility Institutional Animal Care and Use Committee before the initiation of such procedures.

If an animal is determined to be in overt pain/distress, or appears moribund and is beyond the point where recovery appears reasonable, the animal will be euthanized for humane reasons in accordance with the *American Veterinary Medical Association (AVMA) Guidelines on Euthanasia* and with the procedures outlined in the protocol.[4]

By approving this protocol, the Sponsor affirms that there are no acceptable non-animal alternatives for this study, that this study is required by a relevant government regulatory agency(ies) and that it does not unnecessarily duplicate any previous experiments.

24. REFERENCES

1. National Research Council. *Guide for the Care and Use of Laboratory Animals*. 8th edition. Washington, DC: National Academy Press. 2011.
2. Buehler EV. Delayed Contact Hypersensitivity in the Guinea Pig. Arch Dermat. 1965;91:171-177.
3. Office of Laboratory Animal Welfare. *Public Health Services Policy on Humane Care and Use of Laboratory Animals*. Bethesda, MD: National Institutes of Health. August 2002.
4. American Veterinary Medical Association. *AVMA Guidelines on Euthanasia*. February 2013.

25. TESTING FACILITY APPROVAL

The signature below acknowledges Testing Facility Management's responsibility to the study as defined by the relevant GLP regulations.

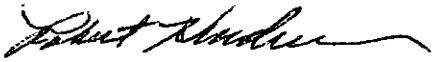
Mark A. Morse Date: 15 SEP 2014
Mark A. Morse, PhD, DABT
Testing Facility Management

The signature below indicates that the Study Director approves the study protocol.

Jason W. Smedley Date: 15 Sep 2014
Jason W. Smedley, BS
Study Director

26. SPONSOR APPROVAL

The protocol was approved by the Sponsor by email on 15 Sep 2014. The signature below confirms the approval of the protocol by the Sponsor Representative.



Date: 15 September 2014

Robert Hinderer, PhD
Sponsor Representative

charles river

PROTOCOL AMENDMENT NO. 1

A Sensitization Study of OS329036A by Dermal Administration in Guinea Pigs-Modified Buehler Design

Testing Facility Study No. 20061359

Note: Additions are indicated in bold text. Deletions are indicated in strikethrough text.

1. Section 13. Experimental Design

Experimental Design-Range-Finding Study

Site No.	Test Material	Dose Level	Number of Animals[a]	
			Males	Females
1	OS329036A	100%	2	2
2	OS329036A	75%		
3	OS329036A	50%		
4	OS329036A	25%		

[a] Additional animals may be necessary depending on the results obtained at each level.

Experimental Design- 2nd Range-Finding Phase

Site No.	**Test Material**	**Dose Level[b]**	**Number of Animals[a]**	
			Males	**Females**
1	**OS329036A**	**35%**	**2**	**2**
2	**OS329036A**	**25%**		
3	**OS329036A**	**15%**		
4	**OS329036A**	**5%**		

[a] Additional animals may be necessary depending on the results obtained at each level.

Experimental Design-Main Study

Group	Phase/Treatment				Number of Animals[a]	
	Induction 1 to 3	Challenge	Rechallenge[a]	Second Rechallenge[b]	Males	Females
Test	Test Substance	Test Substance	Test Substance	Test Substance	10	10
Challenge Control	-	Test Substance	-	-	5	5
Rechallenge Control	-	-	Test Substance	-	5	5
Second Rechallenge Control	-	-	-	Test Substance	5	5
HCA Test	5.0% HCA	2.5% and 1.0% HCA	-	-	5	5
HCA Control	-	2.5% and 1.0% HCA	-	-	5	5

- = not applicable.
[a] To be conducted only if needed to clarify the primary challenge results.
[b] To be conducted only if needed to clarify the rechallenge results.

Justification(s):

A second range-finding phase will be added to determine appropriate challenge concentrations.

2. Section 15. Terminal Procedures-Range-Finding Study

Terminal procedures are summarized in the following table:

Terminal Procedures for Range-Finding Animals

Number of Animals		Scheduled Euthanasia Day	Necropsy Procedures	
M	F		Necropsy	Tissue Collection
2	2	2	-	-
Unscheduled Deaths			X	-

X = procedure to be conducted; - = not applicable.

Terminal Procedures for 2nd Range-Finding Phase

Number of Animals		**Scheduled Euthanasia Day**	**Necropsy Procedures**	
M	**F**		**Necropsy**	**Tissue Collection**
2	**2**	**2**	**-**	**-**
Unscheduled Deaths			**X**	**-**

X = procedure to be conducted; - = not applicable.

Justification(s):

To specify terminal procedures for the 2nd Range-Finding Phase.

Amendment Approval:

Date: 17 Oct 2014

Jason W. Smedley, BS
Study Director

Appendix 2
Test Substance Characterization

The Lubrizol Corporation

Research Testing Laboratory
1275 Lloyd Road
Wickliffe, OH 44092
(440) 943-4200

Certificate of Analysis for OS329036A

Shipment to: CHARLES RIVER LAB C/O FORMULTION DEPT 640 N ELIZABETH ST SPENCERVILLE OH 45887		**Attn:** BETH HOOVER	
Order No.:	2194283	**Date:**	September 22, 2014
Physical Container:	1 x 8 oz	**Retest After:**	2 years from receipt
Batch and Identity Number:	OS329036A		
Purity:	100% product		
NMR/IR:	Structural identity confirmed		
Visual examination:	Viscous Brown Liquid		

By:	Marilyn Fox
Signature:	Marilyn Fox
Customer Service	

1. The results listed in this document are only pertinent to the sample listed.
2. This report should not be reproduced, except in its entirety.
3. Deviations from, additions to, or exclusions from the data portrayed have been described above where appropriate.

Appendix 3
Dermal Grading System

ERYTHEMA AND EDEMA OBSERVATIONS		
OBSERVATION	**DEFINITION**	**CODE**
Erythema - Grade 0	No reaction	0
Erythema - Grade ±	Slight patchy erythema	±
Erythema - Grade 1	Slight, but confluent or moderate patchy erythema	1
Erythema - Grade 2	Moderate, confluent erythema	2
Erythema - Grade 3	Severe erythema with or without edema	3
Maximized Grade 3	Notable dermal lesions	M-3 (see below)
Edema - Grade 1	Very slight edema (barely perceptible)	ED-1
Edema - Grade 2	Slight edema (edges of area well defined by definite raising)	ED-2
Edema - Grade 3	Moderate edema (raised approximately 1 millimeter)	ED-3
Edema - Grade 4	Severe edema (raised more than 1 millimeter and extends beyond the area of exposure)	ED-4

NOTE: An erythema code was assigned to each test site. An edema code was assigned only if edema was present at the test site. If notable dermal lesion(s) (> Grade 1) were present, then the "Maximized Grade 3" was assigned to the test site in place of the erythema score and the type of the notable dermal lesion(s) was noted (e.g., M-3^{ES-2}).

NOTABLE DERMAL LESIONS		
OBSERVATION	**DEFINITION/EXPLANATION**	**CODE**
Eschar	A crust-like formation within or on the test area. Characterized as scab-like (dried blood or lymph) or dead layers of tissue/crust. The area is hardened to the touch and not very pliable. Note: Because erythema cannot be observed through eschar and eschar is considered to be a notable dermal lesion, the erythema score was maximized when eschar was present greater than ES-1. The test site was observed for reversibility in order to determine if the eschar was an in-depth injury. Coded using an area designation (see below).	--
Eschar - Grade 1	Focal and/or pinpoint areas up to 10% of test site	ES-1
Eschar - Grade 2	> 10% < 25% of test site	ES-2
Eschar - Grade 3	> 25% < 50% of test site	ES-3
Eschar - Grade 4	> 50% of test site	ES-4
Blanching	Characterized by areas of white to yellow or tannish discoloration in the test site due to a decreased blood flow to the skin. Note: An erythema score cannot be determined and blanching is considered a notable dermal lesion; therefore, the erythema score was maximized when blanching was present greater than BLA-1. The test site was observed for reversibility in order to determine if the blanching was an in-depth injury. Coded using an area designation (see below).	--
Blanching - Grade 1	Focal and/or pinpoint areas up to 10% of the test site	BLA-1
Blanching - Grade 2	> 10% < 25% of test site	BLA-2
Blanching - Grade 3	> 25% < 50% of test site	BLA-3
Blanching - Grade 4	> 50% of test site	BLA-4
Ulceration	An open lesion in the skin possibly due to the exfoliation of necrotic tissue or eschar formation. Characterized by a crater-like area which is generally inflamed and has a moist exudate. The erythema score was maximized when ulceration was present greater than U-1. Ulceration is considered an in-depth injury. Coded using an area designation (see below).	--
Ulceration - Grade 1	Focal and/or pinpoint areas up to 10% of the test site	U-1
Ulceration - Grade 2	> 10% < 25% of test site	U-2
Ulceration - Grade 3	> 25% < 50% of test site	U-3
Ulceration - Grade 4	> 50% of test site	U-4

NOTABLE DERMAL LESIONS		
OBSERVATION	**DEFINITION/EXPLANATION**	**CODE**
Necrosis	The apparent death of a portion of tissue which may result in irreversible damage depending on the severity of injury based on the color, area and texture. It is characterized by a dark (ranging from gray to black) and often in-depth discoloration of the tissue. Because this term is considered to be diagnostic, this observation was only made with the approval of the Study Director and accompanied by a full description (the color noted). The erythema score was maximized when necrosis was present greater than NEC-1. Necrosis is considered a notable dermal lesion and an in-depth injury. Coded using an area designation (see below).	--
Necrosis - Grade 1	Focal and/or pinpoint areas up to 10% of the test site	NEC-1 (color)
Necrosis - Grade 2	> 10% < 25% of test site	NEC-2 (color)
Necrosis - Grade 3	> 25% < 50% of test site	NEC-3 (color)
Necrosis - Grade 4	> 50% of test site	NEC-4 (color)

ADDITIONAL DERMAL OBSERVATIONS		
OBSERVATION	**DEFINITION/EXPLANATION**	**CODE**
Desquamation or Skin Flaking	Characterized by scaling or flaking of dermal tissue with or without denuded areas. May consist of a range from dry flaking of the skin to more pronounced flaking with denuded areas (in these cases the affected area may have a slight harder "feel" to it as compared to normal tissue; however, this should not be confused with a notable dermal lesion such as eschar). Areas of eschar were not scored for desquamation/skin flaking. This finding is generally not considered significant if the test site is otherwise clear for erythema, edema, etc.	DES or SFLA
Fissuring	Characterized by cracking of the skin or eschar formation (slough and/or scab) that is associated with moist exudate. Fissuring was checked prior to removing the animal from the cage and manipulating the test site.	FIS
Eschar Exfoliation	The process by which areas of eschar flake off the test site. This observation was noted only with an ES observation. May be graded with the following criteria:	EXF
Eschar Exfoliation – Grade 1	Barely perceptible scales.	EXF-1
Eschar Exfoliation – Grade 2	Distinct scales.	EXF-2
Eschar Exfoliation – Grade 3	Pronounced flaking with denuded sites.	EXF-3
Test Site Staining or Skin Staining	Skin located at the test site appears to be stained/discolored possibly due to test substance (note color of staining).	TSS (color) or SSTA
Erythema Extends Beyond the Test Site or Skin Red	The erythema extends beyond the test site. May be referred to as "Skin Red" with an appropriate location. Note: A Study Director was contacted for erythema extending beyond the test site.	ERB or SRED

ADDITIONAL DERMAL OBSERVATIONS		
OBSERVATION	**DEFINITION/EXPLANATION**	**CODE**
Superficial Lightening or Skin Pale	Characterized by pale area(s) (almost a burn-like appearance) in the test site. However, erythema may still be observed through the pale area. Note: This observation may affect the overall erythema score of the test site. This observation may progress to other observations resulting in notable dermal lesions, but by itself was not considered a notable dermal lesion that resulted in a maximized dermal score. May be graded with the following criteria:	SL or SPAL
Superficial Lightening - Grade 1	Focal and/or pinpoint areas up to 10% of the test site	SL-1
Superficial Lightening - Grade 2	> 10% < 25% of test site	SL-2
Superficial Lightening - Grade 3	> 25% < 50% of test site	SL-3
Superficial Lightening - Grade 4	> 50% of test site	SL-4

Appendix 4
Individual Body Weight Data

STUDY NO. 20061359 PAGE 1

APPENDIX 4

A DERMAL SENSITIZATION STUDY IN GUINEA PIGS

INDIVIDUAL BODY WEIGHT DATA

		BODY WEIGHT (G)		
GROUP	ANIMAL NO./SEX	DAY -1	DAY 27	DAY 34
TEST	6292/M	359	516	572
	6293/M	378	611	674
	6294/M	434	623	561
	6295/M	403	544	491
	6296/M	383	606	639
	6297/M	391	565	602
	6298/M	426	617	667
	6299/M	413	584	627
	6300/M	429	566	548
	6301/M	396	551	548
	6336/F	395	501	543
	6337/F	378	527	534
	6339/F	397	600	588
	6340/F	377	542	519
	6341/F	402	571	604
	6342/F	367	485	505
	6343/F	365	545	516
	6344/F	382	519	570
	6345/F	377	568	533
	6346/F	392	513	507
HCA TEST	6302/M	443	690	-
	6303/M	382	531	-
	6304/M	415	613	-
	6305/M	388	527	-
	6306/M	372	448	-
	6347/F	377	514	-
	6348/F	364	490	-
	6349/F	392	546	-
	6350/F	390	550	-
	6351/F	351	453	-

NOTE: - = NOT APPLICABLE.

A DERMAL SENSITIZATION STUDY IN GUINEA PIGS

INDIVIDUAL BODY WEIGHT DATA

		BODY WEIGHT (G)		
GROUP	ANIMAL NO./SEX	DAY -1	DAY 27	DAY 34
CHALLENGE	6307/M	403	594	-
CONTROL	6308/M	443	610	-
	6309/M	443	702	-
	6310/M	335	623	-
	6312/M	441	549	-
	6352/F	388	544	-
	6353/F	397	589	-
	6354/F	362	579	-
	6355/F	349	550	-
	6356/F	395	600	-
HCA CHALLENGE	6313/M	441	645	-
CONTROL	6314/M	400	626	-
	6315/M	435	642	-
	6316/M	391	567	-
	6317/M	410	589	-
	6357/F	401	534	-
	6358/F	351	478	-
	6359/F	379	531	-
	6360/F	368	510	-
	6361/F	388	600	-
RECHALLENGE	6318/M	372	-	718
CONTROL	6320/M	421	-	704
	6321/M	393	-	606
	6322/M	387	-	611
	6323/M	408	-	622
	6362/F	363	-	530
	6363/F	371	-	594
	6365/F	350	-	552
	6366/F	359	-	571
	6367/F	377	-	630

NOTE: - = NOT APPLICABLE.

A DERMAL SENSITIZATION STUDY IN GUINEA PIGS

INDIVIDUAL BODY WEIGHT DATA

GROUP	ANIMAL NO./SEX	BODY WEIGHT (G) DAY -1	DAY 27	DAY 34
SECOND	6324/M	442	-	-
RECHALLENGE	6325/M	389	-	-
CONTROL	6326/M	401	-	-
	6327/M	403	-	-
	6330/M	385	-	-
	6368/F	368	-	-
	6369/F	379	-	-
	6370/F	367	-	-
	6371/F	385	-	-
	6372/F	397	-	-

NOTE: - = NOT APPLICABLE.
[a]A SECOND RECHALLENGE CONTROL GROUP WAS MAINTAINED ON STUDY; HOWEVER, THE SECOND RECHALLENGE PROCEDURE WAS NOT REQUIRED AS THE RECHALLENGE RESULTS WERE DEFINITIVE.

Appendix 5
Individual Clinical/Necropsy Observations

APPENDIX 5

A DERMAL SENSITIZATION STUDY IN GUINEA PIGS

INDIVIDUAL CLINICAL/NECROPSY OBSERVATIONS

GROUP/PHASE	ANIMAL NO./SEX	CLINICAL OBSERVATION	NECROPSY OBSERVATION
HCA CHALLNGE CONTROL/CHALLENGE	6358/F	FOUND DEAD	SKIN: MATERIAL ACCUMULATION, DARK, AROUND NOSE, MOUTH, AND RIGHT FOOT LUNG: DISCOLORATION, DARK, ALL LOBES AND FAILURE TO COLLAPSE, ALL LOBES

ORIGIN ID:LNNA (440) 347-5004
DAWN EVANGELISTA
LUBRIZOL
29400 LAKELAND BLVD

WICKLIFFE, OH 44092
UNITED STATES US

SHIP DATE: 16JAN15
ACTWGT: 1.0 LB MAN
CAD: 0920848/CAFE2806

BILL SENDER

TO **TSCA CONFIDENTIAL BUIS. INFO. 7407M**
EPA EAST ROOM 6428 ATN:SECTION 8(E)
U.S. ENVIRONMENTAL PROTESTION AGENC
1200 PENNSYLVANIA AVENUE
WASHINGTON DC 20460
(202) 564-8940
REF: CO1203/MAIL

FedEx Express

TRK# 0201 **6064 9187 4611**

MON - 19 JAN AA
STANDARD OVERNIGHT

XC RDVA

20460
DC-US **IAD**